Status Active PolicyStat ID 13057818

Beaumont

Origination	3/2/2023
Last Approved	3/2/2023
Effective	3/2/2023
Last Revised	3/2/2023
Next Review	3/1/2025

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Applicability	Royal Oak

Histology Muscle Enzyme - Myoadenylate Deaminase - Royal Oak

Document Type: Procedure

I. PURPOSE AND OBJECTIVE:

This procedure is used to stain a type of oxidoreductase enzyme known as myoadenylate deaminase. This oxidative enzyme catalyzes the reaction between the substrate and oxygen. It is found in the mitochondria in normal muscle. Absence of this enzyme is associated with muscle fatigue and exercise induced pain. This stain does show a difference in muscle fiber types but should not be used primarily for fiber typing. Type I shows blue granular densities on a clear cytoplasmic background, whereas Type II fibers show a finer reticular blue staining on a diffusely pink cytoplasm. This stain can also be used to demonstrate tubular aggregates (aggregates of sarcoplasmic reticulum tubules).

II. PRINCIPLE:

The reaction is an alkaline tetrazolium reaction, where the tetrazolium salt (NBT) is reduced to a formazan pigment by a thiol at an alkaline pH. The substrate sodium adenylate (AMP), at pH 6.1, is hydrolyzed by AMP to inosinic acid (IMP) plus ammonia. This ammonia, at the sites of enzyme activity, will raise the pH by a few tenths (to 6.4). At this pH, the dithiothreitol reduces the tetrazolium salt (nitro blue tetrazolium (NBT)) to a formazan pigment, which is blue. Away from the sites of enzyme activity, the ammonia is neutralized by the buffer, so there is no general increase in pH, and therefore no generalized staining. The potassium chloride retards the formazan formation, while at the same time activates the adenylate deaminase, thereby increasing specificity of the reaction. Sodium hydroxide is used to establish a pH of 6.1.

AMP + myoadenylate deaminase → isosinic acid (IMP) + ammonia

ammonia + dithiothreitol → increases pH to 6.4 → reduced NBT
reduced NBT → formazan precipitate (blue)

III. SPECIMEN COLLECTION AND HANDLING:

A. Fixation
    1. Unfixed tissue that has been frozen.

B. Processing
    1. Fresh tissue.
    2. No processing.

C. Section Thickness
    1. Cut frozen sections at 8-12µ.

D. Slide Drying
    1. None.

E. Type of slide
    1. Plus slides.

IV. REAGENTS:

A. **3M Potassium Chloride**

Potassium chloride	**22.4gm**
Distilled water	**100.0 ml**

Dissolve together. May be stored at room temperature; stable for months.

B. **0.02 M Sodium Hydroxide**

Sodium hydroxide	**0.8 gm**
Distilled water	**1000.0 ml**

Slowly add sodium hydroxide to water. Stir until dissolved. May be stored at room temperature; stable for months.

C. **1N Hydrochloric Acid**

Hydrochloric acid	**3.7 ml**
Distilled water	**96.3 ml**

Slowly add hydrochloric acid drop by drop, to water. Mix together. Store at room temperature; stable for months.

D. **Rinse Buffer**

Potassium chloride	**11.18 gm**
Sodium citrate	**0.441 gm**
Distilled water	**1000.0 ml**

Dissolve together with stirring. pH to 6.0 with 1N hydrochloric acid. Store at room temperature; stable for months.

E. **Solution A**

Nitro blue tetrazolium (NBT)	**0.010 gm**

Sodium adenylate (adenosine monophosphate (AMP)) 0.004 gm
Distilled water 9.0 ml

JUST BEFORE USE, dissolve together. When dissolved, slowly add:

3M potassium chloride 0.7 mL

When completely mixed, adjust pH to 6.1 with 0.02M sodium hydroxide.

F. **Solution B**

Dithiothreitol 0.005 gm
Distilled water 0.3 ml

JUST BEFORE USE, dissolve together. DO NOT pH; harmful to pH probe.

G. **Incubating Medium**

JUST BEFORE USE, add Solution B drop by drop to Solution A, swirling to mix.

V. EQUIPMENT:

A. Mettler balance
B. 37°C oven

VI. SUPPLIES:

A. Erlenmeyer flasks
B. Graduated cylinders
C. Coplin jars
D. Pipets
E. Filter paper

VII. SPECIAL SAFETY PRECAUTIONS:

A. Potassium Chloride
    1. Is an irritant.
B. Sodium Hydroxide
    1. Is a corrosive.
    2. Add carefully to water.
    3. May cause severe skin and eye burns.
C. Hydrochloric Acid
    1. Is an acid.
    2. Add slowly, drop by drop, to water.
    3. May cause severe eye and skin burns.
D. Sodium Citrate
    1. Has low hazard for recommended handling.

E. Nitroblue Tetrazolium
    1. Is an irritant.

F. Sodium Adenylate (AMP)
    1. Not a hazardous substance.
    2. Must be kept refrigerated.

G. Dithiothreitol
    1. Is harmful if absorbed through the skin or swallowed.
    2. May cause skin burns. Irritant to respiratory system and eyes.
    3. Must be kept FROZEN.
    4. Is harmful to pH meter probes.

VIII. QUALITY CONTROL:

Frozen section of muscle with nerve.

IX. LIMITATIONS:

A. Solution "A", adjust the pH BEFORE the addition of dithiothreitol. The dithiothreitol is harmful to pH probes.
B. Dithiothreitol must be kept frozen for storage to prevent deterioration.
C. NBT must be kept refrigerated.
D. This enzyme is very sensitive to fixation. Avoid all types of fixatives.
E. Fiber-typing must be based on color differences rather than on intensity differences.

X. PROCEDURE:

Step	Action	Time	Notes
1	Pour incubating solution over slides.		**Cover to prevent evaporation. Incubating medium must be made up JUST BEFORE USE.**
2	Incubated at room temperature.	1 hour	**Incubation time may need to be varied for each muscle, as the staining rate will increase with an increase in pH, dithiothreitol, and tetrazolium.**
3	Rinse with rinse buffer, 2 changes	5-10 seconds each	
4	Rinse with 30% Acetone, 60% Acetone, 30% Acetone.	10 seconds each	
5	Dehydrate through graded alcohols, clear with		

	xylene.		
6	Coverslip using a synthetic mounting media.		

XI. RESULTS:

A. Mitochondria - **blue**

B. Type I fibers - **granular blue densities on a clear cytoplasmic background**

C. Type II fibers - **finer reticular blue staining on diffusely pink cytoplasm**

XII. REFERENCES:

A. Bancroft JD, Stevens A: Theory and Practice of Histological Techniques, 3rd ed. New York, NY, Churchill Livingstone, 1990.

B. Brumback RA, Leech RW: Color Atlas of Muscle Histochemistry, PSG Publishing Company, Inc., Littleton, MA, 1984.

C. Fishbein WN, Griffin JL, Armbrustmacher VW: Stain for Skeletal Muscle Adenylate Deaminase. Arch Pathol Lab Med. 104:462, 1980.

Approval Signatures

Step Description	Approver	Date
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Applicability

Royal Oak